



US006080553A

**United States Patent** [19]

Sogabe et al.

[11] **Patent Number:** **6,080,553**[45] **Date of Patent:** **\*Jun. 27, 2000**[54] **CREATINE AMIDINOHYDROLASE,  
PRODUCTION THEREOF AND USE  
THEREOF**3,907,644 9/1975 Mollering et al. .... 195/99  
5,451,520 9/1995 Furukawa et al. .... 435/227[75] **Inventors:** Atsushi Sogabe; Takashi Hattori;  
Yoshiaki Nishiya; Yoshihisa  
Kawamura, all of Isuruga, Japan[73] **Assignee:** Toyo Boseki Kabushiki Kaisha,  
Osaka, Japan[\*] **Notice:** This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).[21] **Appl. No.:** 08/799,897[22] **Filed:** Feb. 13, 1997[30] **Foreign Application Priority Data**

Feb. 13, 1996 [JP] Japan ..... 8-025435

[51] **Int. Cl.<sup>7</sup>** ..... C12Q 1/34; C12N 9/78;  
C12N 1/20; C12N 1/00[52] **U.S. Cl.** ..... 435/18; 435/227; 435/192;  
435/252.3; 435/320.1; 435/829; 435/252.33[58] **Field of Search** ..... 435/18, 227, 252.1,  
435/320.1, 829, 192, 252.3, 252.33[56] **References Cited****U.S. PATENT DOCUMENTS**

3,806,420 4/1974 Holz et al. .... 195/66

**FOREIGN PATENT DOCUMENTS**62 091182 4/1987 Japan .  
07 265074 10/1995 Japan .*Primary Examiner*—Elizabeth Slobodyansky  
*Attorney, Agent, or Firm*—Leydig, Voit & Mayer, Ltd.[57] **ABSTRACT**

A creatine amidinohydrolase having the following physico-chemical properties:

Action: catalyzing the following reaction;  
creatine+H<sub>2</sub>O→sarcosine+urea

Optimum temperature: about 40–50° C.

Optimum pH: pH about 8.0–9.0

Heat stability: not more than about 50° C. (pH 7.5, 30 min)

Km value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: about 3.5–10.0 mM

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 3.5,

a method for producing said enzyme, comprising culture of microorganism producing said enzyme, a method for the determination of creatine or creatinine in a sample using said enzyme, and a reagent therefor.

**23 Claims, 2 Drawing Sheets**

09940941-082801